EFFECT OF DIET ON EYE-COLOR DEVELOPMENT IN DROSOPHILA MELANOGASTER[1]

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Vermilion brown (*v bw*) larvae of *D. melanogaster* placed on a low food level diet produce v^+ eye-color hormone, and therefore, as flies, develop pigmented eyes (Khouvine, Ephrussi and Chevais, 1938). Beadle, Tatum and Clancy (1938) showed that larvae are affected in this way by low food level during a certain sensitive period lying between 60 and 70 hours from egg laying. Khouvine, Ephrussi and Chevais reported that sugar added to the starvation diet inhibits the starvation effect. Their work, however, did not eliminate the possibility that the sugar effect was associated only indirectly with hormone production in the flies, possibly through the intermediation of growing yeast or other micro-organisms. We have investigated the effects under aseptic conditions of various supplements to a low yeast diet on the growth and eye-color development of vermilion brown animals. Under these conditions carbohydrates and related substances inhibit the starvation effect, while proteins and amino acids do not. The present paper summarizes these results.

Experimental

Culture and Methods

The aseptic cultures of vermilion brown larvae used throughout this work were obtained by a slight modification of Baumberger's (1919) alcohol sterilization method. Eggs were collected over a 2- to 3-hour period on freshly autoclaved standard corn-meal molasses agar, without added yeast. Shortly after collection 20 to 30 eggs were picked up on a single small sterilized glass rod flattened at the end. The rods with the eggs were then placed individually in small sterile vials containing 85 per cent alcohol. After 10 minutes the rod was removed and the eggs were pushed off onto the sterile test medium, using ordinary bacteriological methods to insure sterility. All cultures were incubated at 25° C. unless otherwise stated.

The standard starvation food contained 1.5 per cent agar and 0.5 per cent Fleischmann's dry brewers' yeast made up with distilled water.

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Ten cc. of this mixture and the desired amounts of the various supplements were placed in 35 cc. vials which were stoppered with cloth-covered cotton plugs. After sterilization in the autoclave the vials were cooled and agitated, and finally slanted so that the solid yeast remained suspended throughout the medium. Routine checks of sterility were made after pupation of the larvae by streaking a loopful of the medium onto yeast extract-glucose agar. Any vials which were not bacteriologically sterile at this time were discarded.

Cultures were observed every 24 hours, so that the time to emergence of the flies was accurate only within this period. The observed prolongation of larval and pupal life as compared with the normal 215 hours on full food is given in days from egg-laying. The delay actually represents prolongation of larval life, since Beadle et al. (1938) showed

TABLE I

Influence of yeast concentration and temperature on the starvation effect. Basic medium: 1.5 per cent agar.

Temperature °C.	Yeast concentration (per cent)											
	0.5			1.0			3.0			5.0		
	Days to emergence	No. of adult flies	Eye color	Days to emergence	No. of adult flies	Eye color	Days to emergence	No. of adult flies	Eye color	Days to emergence	No. of adult flies	Eye color
17°	22–25*	7	3.5–4.5	27–29	22	3.5–5.0	24–26	39	1.0–3.0	24–26	35	0.5–2.5
25°	11–13	33	2.5–3.5	10–11	15	0.5–1.5	10	20	0.0–0.2	9–10	42	0.0
28°	10–11	26	0.5–1.2	9–10	19	0.0	8–9	29	0.0	8–9	34	0.0

* Normal developmental time on full food is 9 days (215 hours) from egg-laying.

that duration of pupal life is practically constant under all conditions. After emergence of the flies the intensity of pigmentation of the eyes was graded according to the scale of eye-color values described by Tatum and Beadle (1938). These values have a definite relation to the amount of hormone available to the fly, but for simplification all results are given only as color values. It should be remembered that the increased intensity of eye pigmentation resulting from starvation involves the actual production of v^+ hormone (Beadle et al., 1938).

Effect of Yeast Concentration and Temperature

In order to determine the most suitable conditions for the starvation effect, series with varying yeast concentrations were incubated at different temperatures, 18°, 25° and 28° C. The results are given in Table I. It was found that 0.5 per cent dry yeast at 25° C. was most

TABLE II

Influence of carbohydrates on the starvation effect. Basic medium: 0.5 per cent brewers' yeast in 1.5 per cent agar.

	Carbohydrates added (2 per cent concentration)			
	None	Starch	Sucrose	Glucose
Delay in days	2–3	2–5	1–4	1–4
Number of flies	9	62	67	45
Eye color	2.0–3.5	0.0–0.5	0.0–0.3	0.1–1.0

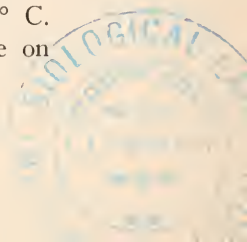
suitable, both for the intensity of the effect and for the developmental time required. Lower concentrations of yeast at this temperature gave somewhat stronger effects, but mortality was higher. The higher temperature, 28° C., speeded up development and greatly decreased the in-

TABLE III

Influence of sucrose concentration on eye-color (starvation effect) and length of larval life. Basic medium: 0.5 per cent brewers' yeast in 1.5 per cent agar. (Figures in parenthesis indicate number of flies.)

Prolongation of larval life	Sucrose concentration (*per cent*)							
	0	0.05	0.1	0.3	0.5	1.0	2.0	4.0
days 1			4.0–4.5 (4)	1.5–2.0 (21)	1.0–2.5 (60)	0.3–1.5 (22)		
2	2.0–3.5 (2)	4.0 (2)	3.5 (17)	3.5 (19)	2.5–3.0 (18)	0.5–1.5 (21)	0.0–0.2 (11)	
3	2.0–3.5 (7)	3.5 (20)	3.0 (10)	3.0 (7)	2.0–3.0 (6)	0.0–1.5 (8)	0.0–0.6 (19)	
4	2.0–4.0 (17)	2.0–3.0 (5)	3.0 (5)	2.5 (2)			0.0–0.5 (5)	0.0 (2)
5	2.5–3.5 (2)	1.5 (1)					0.0–0.8 (5)	0.0 (20)
Total	2.0–4.0 (28)	1.5–4.0 (28)	3.0–4.5 (36)	1.5–3.5 (49)	1.0–3.0 (84)	0.0–1.5 (51)	0.0–0.8 (40)	0.0 (22)

tensity of the starvation effect; i.e., pigment production did not take place on yeast concentrations over 0.5 per cent. At 25° C., 3 per cent yeast or more prevented the starvation effect, while pigment appeared on all concentrations up to and including 5 per cent yeast at 17° C. This effect of temperature may be due to a differential influence on



larval activity (food intake) and on the rate of metabolic processes. The medium containing 0.5 per cent yeast was selected for standard starvation and used throughout further work. At 25° C. it consistently delayed larval development from 2 to 4 days and gave eye-color values of from 2.5 to 3.5. This is equivalent to a v^+ hormone production of 3.5 to 8.0 units per individual (Tatum and Beadle, 1938). Controls were made for each series of experiments, with similar results. These control starvation values are omitted from the tables in most cases.

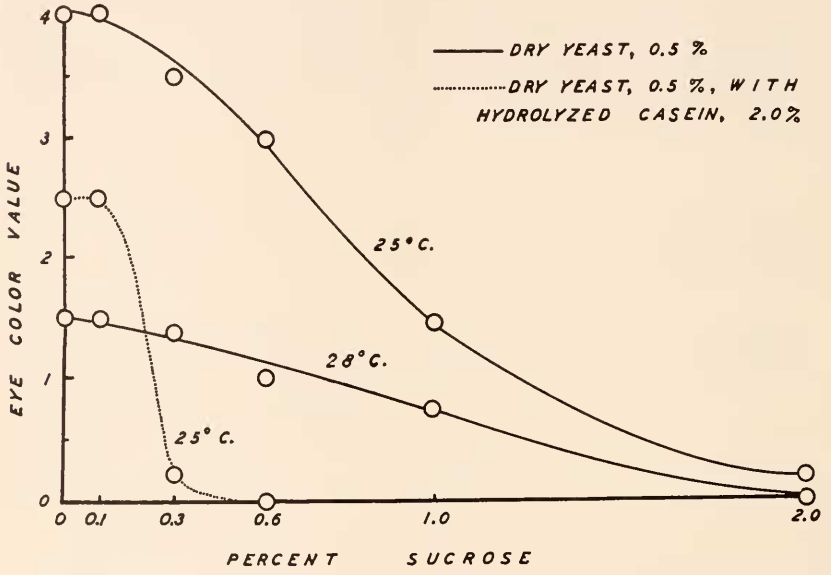


FIG. 1. Influence of temperature and of hydrolyzed casein on sucrose inhibition of the starvation effect (maximum color values for each sugar concentration used in plotting curves). Basic medium: 0.5 per cent brewers' yeast in 1.5 per cent agar.

Effect of Carbohydrates

Table II shows the influence of added carbohydrates on the starvation effect. Starch, sucrose, and glucose almost completely inhibited the production of pigment, although larval life was prolonged as much as or more than in the controls without carbohydrate. Several series of experiments were made to establish the relation of sugar concentration to prolongation of larval life and intensity of the starvation effect. Table III gives the combined results of these series. Concentrations of sucrose up to 0.5 per cent shortened larval life as compared to the control, without very marked effect on eye pigmentation. Higher sucrose concentrations progressively prolonged larval life and inhibited pig-

mentation. Two per cent sucrose caused about the same delay as in the control, but almost completely prevented v^+ hormone production. Four per cent sucrose seemed to be toxic and prolonged larval life even more than in the control, but completely suppressed the starvation effect.

The inhibiting effect of varying concentrations of sugar at 28° C. was also determined. The influence of temperature on the sucrose effect is shown in Fig. 1. It required almost the same concentration of sugar (2 per cent) to inhibit completely pigmentation at the higher temperature as at 25° C., although the production of pigment on the control starvation food without sugar was much less at 28° C.

TABLE IV

Influence of other substances on the starvation effect. Basic medium: 0.5 per cent brewers' yeast in 1.5 per cent agar.

Substance added	Prolongation of larval life in days	Number of adult flies	Eye color
Sodium benzoate, 1 per cent*	1–3	20	0.1–1.5
Sodium benzoate, 1 per cent†	2–3	5	0.5–0.8‡
Calcium acetate, 2 per cent	6–9	30	0.0–0.2
Calcium lactate, 3 per cent	5–9	30	1.0–3.0
Calcium carbonate, 2 per cent	6–9	20	2.0–3.5
Ethyl alcohol, 5 per cent§	1–5	22	0.0–0.1
Glycerol, 2 per cent	3–7	38	2.0–3.0
Butter fat, 4 per cent	3–8	11	0.0–0.2

* Sterile 60-hour-old fully fed larvae transferred aseptically to test medium.
† Eggs not sterilized.
‡ Control with no benzoate; color = 3.0.
§ Alcohol added after cooling medium to 35°C.

The results of these experiments with carbohydrates show that prolongation of larval life is not necessarily accompanied by v^+ hormone and eye pigment production. However, the starvation effect is observed only when larval life is prolonged.

Influence of Other Substances on the Starvation Effect

It seemed possible that some indication of the nature of the starvation effect might be obtained by similarly testing substances other than carbohydrates. Table IV summarizes the results of these experiments. Calcium lactate, calcium carbonate, and glycerol had only very slight inhibiting effects on pigmentation. On the other hand, ethyl alcohol, butter fat, and calcium acetate prevented the starvation effect almost completely. Sodium benzoate was quite toxic, but under non-lethal conditions it prevented pigment production to a considerable degree.

Each of these various additions to the starvation diet considerably prolonged larval life, but production of v^+ hormone was suppressed only by certain specific substances, all of which, with the exception of sodium benzoate, may be assumed to be metabolized in a manner similar to carbohydrates. No explanation can be suggested for the inability of glycerol and calcium lactate to function in this way. Concentrations of calcium lactate and glycerol from 0.5 to 3.0 per cent have been used with similar results in every concentration.

Effect of Proteins and Amino-acids

In contrast to carbohydrates, which definitely inhibit the starvation effect, whole and hydrolyzed proteins and mixtures of amino acids, including tryptophane, have no significant effect in reducing either duration of larval life or pigment production. These results are given in Table V.

TABLE V

Influence of protein and amino acids on starvation effect. Basic medium: 0.5 per cent brewers' yeast in 1.5 per cent agar.

Substance added	Prolongation of larval life in days	Number of adult flies	Eye color
Gelatine, 3 per cent	5–6	2	1.5–2.0
Gelatine, 3 per cent; Tryptophane, 1 per cent	5–9	7	1.5–2.5
Mixture of amino acids*	11	2	3.5
Hydrolyzed casein, 0.5 per cent	2–4	13	2.5–3.5
Hydrolyzed casein, 1 per cent	2–4	18	2.0–3.5
Hydrolyzed casein, 2 per cent	2–4	11	1.0–3.0
Hydrolyzed casein, 4 per cent	5–6	9	0.2–3.0

* Tryptophane, tyrosine, cystine, leucine, asparagine, glycine, alanine; 0.1 per cent each.

Although hydrolyzed casein alone had very little effect on pigmentation, it greatly intensified the sucrose effect. The result of a series containing 2 per cent hydrolyzed casein and increasing amounts of sugar is graphically represented in Fig. 1. In the presence of hydrolyzed casein, a sucrose concentration of 0.3 per cent almost completely inhibited hormone production. The other curves in Fig. 1 give for comparison the effect of sucrose without hydrolyzed casein. In the presence of an excess of amino acids, the sugar concentration effective in pigment inhibition was about that optimal for growth (see Table III).

Discussion

Khouvine et al. (1938) suggested that sugar may have a protein-sparing action, and that the starvation effect and production of v^+ hormone involves an abnormal protein degradation in the larva. It seems possible, from our results, that the action of carbohydrates and similar substances may be due to their protein-sparing action. However, it is probable that other factors are also involved since the sugar concentration optimal for growth does not inhibit the starvation effect and pigment production. This concentration (0. 5 per cent) should have the same protein-sparing action as higher concentrations. In the presence of an adequate supply of amino acids (hydrolyzed casein), however, sugar completely inhibits the starvation effect at the 0.5 per cent concentration optimal for growth.

Carbohydrates seem to inhibit pigment production in starvation by altering the starvation metabolism in such a way that v^+ hormone is not produced, and not by affecting the utilization of the hormone. Khouvine et al. showed that a diet containing sugar did not affect the utilization of ingested v^+ hormone supplied as a *Calliphora* extract. In addition, we have injected mixtures of glucose with extracts containing v^+ substance into *v bw* larvae with no decrease in the effectiveness of the hormone.

Substances other than carbohydrates which also prevent the starvation effect probably act in the same way, since theoretically they may be metabolized in a similar manner. The action of sodium benzoate, since it has no relationship to carbohydrates metabolically, may have a different basis. Sodium benzoate acts similarly to sugar in that it inhibits the production of v^+ hormone by *v bw* larvae on a starvation diet. However, it has no effect on the normal hormone production by su^2-*v, v bw* larvae (normal eye-color ♂ = 1.0; ♀ = 2.0). Nor does sodium benzoate influence the utilization of ingested v^+ hormone by *v bw* larvae.

Beadle et al. (1938) showed that starvation is effective only during a certain sensitive period in larval development. This period was found to lie between 60 and 70 hours of normal development. The starvation effect may be assumed to be a result of prolonging this specific developmental period. Preliminary experiments designed to determine the effect of sucrose on this sensitive period were carried out under aseptic conditions by placing fully fed 54-hour-old larvae on low food with and without sugar. At intervals thereafter larvae were removed from the starvation food to plain agar. The ability to pupate served as the criterion of the end of the 60–70-hour sensitive period (see Beadle et al., 1938). The results seemed to indicate that this period is significantly shortened by sugar in the starvation food.

Whether the action of carbohydrates in inhibiting the starvation effect is due to a direct influence (possibly through a protein-sparing action) on specific processes which during the starvation period lead to the production of v^+ hormone, or whether it is due to a differential acceleration of development during the 60–70-hour sensitive period, thereby shortening the effective time of starvation, cannot be definitely decided at present.

Summary

The production of v^+ eye-color hormone and development of pigment in the double recessive vermilion brown of *D. melanogaster* may be brought about by feeding the larvae on sub-optimal levels of dead yeast under aseptic conditions.

With a given concentration of yeast, culture of larvae at low temperature (17° C.) greatly increases the intensity of the starvation effect. High temperature (28° C.), on the other hand, decreases the intensity of the starvation effect.

Carbohydrates and related substances (acetate, fat, and ethyl alcohol) added to the low yeast diet, under aseptic conditions, completely inhibit the starvation effect by their direct action on larval metabolism and development.

Proteins and amino-acids have very little influence on the starvation effect, but greatly lower the carbohydrate level required to completely inhibit pigment production.

The starvation effect is always associated with prolongation of larval life, but great prolongation of life is possible under certain conditions without any modification of eye color.

The inhibition by carbohydrates may be due to a direct influence on processes proceeding during starvation or to a specific acceleration of development during the period sensitive to starvation, or to both.

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